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## Incidence of *Escherichia coli* O157:H7 in Thailand

(Kejadian *Escherichia coli* O157:H7 di Thailand)

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### ABSTRACT

*Enterohemorrhagic Escherichia coli* (EHEC) especially serotype O157:H7 is one of the important food-borne pathogens because it is able to produce crucial toxins Shiga. However, the outbreak of this organism in Thailand has not been reported. Antibody to O157 antigen was detected in some Thai populations and Shiga toxin-producing *E. coli* were detected in low numbers of clinical specimens. Interestingly, some *E. coli* that showed positive to O157 fimbriae probe and lack of virulence gene were isolated from certain patients and one isolate of *E. coli* O157:H7 which possessed *stx*<sub>1</sub>, *stx*<sub>2v</sub> was detected in a normal child. In addition, the incidence of *E. coli* O157:H7 strains were monitored by the samples from cattle and retail beef in Thailand although their inability to produce toxins or produce in a low concentration was demonstrated. This review discusses the incidences of *E. coli* O157 in clinical and environmental samples of Thailand including the transmission possibility of this bacterium across the Thai border through food trade.

**Keywords:** *E. coli* O157:H7; EHEC; Malaysia; Thailand

### ABSTRAK

*Enterohemorrhagic Escherichia coli* (EHEC) khususnya serotype O157:H7 adalah salah satu patogen hasil makanan yang penting kerana ia dapat menghasilkan toksin penting Shiga. Walau bagaimanapun, wabak organisma ini di Thailand tidak dilaporkan. Antibodi kepada antigen O157 dikesan dalam beberapa populasi Thai dan toksin Shiga yang menghasilkan *E. coli* telah dikesan dalam bilangan yang rendah melalui spesimen klinikal. Menariknya, terdapat beberapa *E. coli* yang menunjukkan kesan positif kepada kuar fimbriae O157 dan kekurangan gen virulen telah diasingkan daripada pesakit tertentu dan satu pengasingan daripada *E. coli* O157:H7 yang mempunyai *stx*<sub>1</sub>, *stx*<sub>2v</sub> dikesan pada kanak-kanak biasa. Di samping itu, kadar strain *E. coli* O157:H7 telah dikawal melalui sampel daripada anak lembu dan daging lembu runcit di Thailand walaupun ketidakmampuan mereka untuk mengeluarkan toksin atau dalam kepekatan rendah telah ditunjukkan. Kajian ini membincangkan kejadian *E. coli* O157 dalam sampel klinikal dan alam sekitar di Thailand termasuk kemungkinan pemindahan bakteria ini merentasi sempadan Thailand melalui perdagangan makanan.

**Kata kunci:** *E. coli* O157:H7; EHEC; Malaysia; Thailand

### INTRODUCTION

*Escherichia coli* is a facultative anaerobic bacterium that exists in the gut of warm blood animals, including human. It plays an important role as a normal microbiota that exerts mutual benefits to the hosts (Drasar & Hill 1974). However, some strains of *E. coli* possess virulence factors and are pathogenic to human. Five pathotypes of *E. coli* have been classified on the basis of their pathogenic mechanisms; enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (EHEC) (Nataro & Kaper 1998). Amongst these five pathotypes, EHEC seems to possess the most devastating effect resulting in death cases reported from several countries (Dundas et al. 2001; Michino et al. 1999; Rangel et al. 2005; Siegler 1995).

EHEC is Shiga toxin-producing *E. coli* (STEC) that possesses *eae* gene (Nataro & Kaper 1998). It is capable of causing hemorrhagic colitis (HC) and the hemolytic

uremic syndrome (HUS) (Karmali et al. 1983; Riley et al. 1983). The major virulence factor of EHEC is the Shiga toxin (Stx) that composed of two major types, Stx1 and Stx2. Stx1 and Stx2 are encoded by *stx*<sub>1</sub> and *stx*<sub>2</sub>, respectively (Griffin 1995). An EHEC isolate may produce *stx*<sub>1</sub> or *stx*<sub>2</sub> or both and it was found that *stx*<sub>2</sub> is about 1000 times more cytotoxic than Stx1 upon human renal endothelial cells (Louise & Obrig 1995). Most of the EHEC isolated from patients are *E. coli* O157:H7 harboring *stx*<sub>2</sub>. The initial description of *E. coli* O157:H7 epidemiology has been documented since the first infection by a rare serotype, O157:H7, in the United States (Karmali et al. 1983; Riley et al. 1983), then, several reports of EHEC O157 have been subsequently demonstrated in the countries all around the globe (Nataro & Kaper 1998). Additionally, detection of *E. coli* O157 in clinical specimens from Asia including India, China, Korea and Hong Kong, have also been reported (Griffin 1995; Gupta et al. 1992; Kim et al. 1998; Yam et al. 1998).

In Thailand, EHEC O157 was isolated from beef and bovine feces collected from local cattle (Sukhumungoon et al. 2011a; Vuddhakul et al. 2000). Moreover, antibody against O157 was detected in some Thai populations (Voravuthikunchai et al. 2005; Vuddhakul et al. 2000). Nevertheless, outbreaks of EHEC O157 have not been reported in Thailand. In this review, the presence of this bacterium in Thailand, the possible reasons explaining the lack of outbreaks of *E. coli* O157 in Thailand, including the meat imported from a neighboring country to Thailand, were discussed.

#### *E. COLI* O157

Many serotypes of EHEC have been demonstrated to cause the diseases but the most striking serotype is O157:H7. It has been reported in several outbreaks of gastrointestinal illnesses in the countries including the United States and Japan (Muto et al. 2008; Rangel et al. 2005). Incidence of outbreaks mostly involved with the consumption of under-cooked beef (Rangel et al. 2005) because the cattle is one of the important EHEC reservoirs (Nataro & Kaper 1998). Currently, the effective approach to isolate *E. coli* O157 is an immunomagnetic separation (IMS) technique using anti O157-coated magnetic beads to capture the bacteria in enrichment broth and subsequently cultivated in appropriate media such as Sorbitol MacConkey agar (SMAC) or CHROMagar O157. Confirmation involves the detection of virulence genes (*stx* and *eae*), including 60-MDa plasmid detection by PCR or DNA hybridization.

Relationship of *E. coli* O157:H7 strains can be investigated by certain molecular tools such as IS-printing and pulsed-field gel electrophoresis (PFGE). IS-printing is a multiplex-PCR which is used to detect insertion sequences, IS629, loci based upon the genome sequence of *E. coli* O157:H7 Sakai strain (Ooka et al. 2009a). This technique can be applied to many O157:H7 strains to determine genetic relationship. In IS-printing, two sets of eighteen primer pairs are targeted to differentiate IS629 loci including *stx*<sub>1</sub>, *stx*<sub>2</sub>, *eae* and *hlyA* genes of *E. coli* O157 strains, generating different length of DNA fragments which subsequently analyzed by high percentage of agarose gel to construct the DNA fingerprint pattern. Nonetheless, pulsed-field gel electrophoresis seems to be a better technique and still be used as a gold standard to investigate the relationship among *E. coli* O157 strains although this technique is laborious and time consuming (Ooka et al. 2009a; Sukhumungoon et al. 2011a).

#### *E. COLI* O157 ANTIBODY DETECTED IN THAI POPULATION

Based on work from Vuddhakul et al. (2000), they investigated the presence of O157 antibody in sera obtained from Thai and Japanese by agglutination assay. O157 antigen used was derived from *E. coli* O157:H7 strain 96-1 that was able to produce both Stx1 and Stx2. In the study, the cut-off titre value for anti-O157 antibody is 10 or more. Five and one Japanese samples

showed titre of 10 and 40, respectively, whereas 18, 3 and 1 Thai samples demonstrated titre of 10, 20 and 40, respectively. This suggests that Thai populations may be exposed to *E. coli* O157 more often than Japanese. Alternatively, other bacterial species that possess O157 antigen may be responsible to raise O157 antibody in Thai people (Bettelheim et al. 1993; Chart et al. 1993). Voravuthikunchai et al. (2005) also investigated IgM and IgG against *E. coli* O157 lipopolysaccharide in healthy blood donor and non-diarrheal patients at Hat-yai Hospital, Songkhla province, Thailand during August 2000 to September 2002 by indirect ELISA. The lipopolysaccharide was prepared from *E. coli* O157:H7 RIMD 0509890, which was able to produce Stx1 and Stx2. With the cut-off value of 2 standard deviation above the mean for each age-group of optical density determined at 405 nm, 39 of 332 subjects (11.7%) and 75 of 332 subjects (22.6%) were positive for IgM and IgG, respectively. When the antibody to O157 level of serum samples obtained from healthy Thai people (21-50 yr) were focused on, it was found that the titre was ranging around 10-40 which was correlated to the work from Vuddhakul et al. (2000).

#### TECHNIQUES USED FOR ISOLATION OF *E. COLI* O157 IN THAILAND

For clinical specimens obtained from diarrheal patients at Bamrasnaradura Hospital, Nonthaburi and Songklanagarind Hospital, Songkhla including specimens taken from diarrheal children from Children's Hospital, Bangkok and 15 different hospitals across Thailand, MacConkey (Mac) and sorbitol MacConkey agar (SMAC) were used to isolate STEC or *E. coli* O157 (Brown et al. 1989; Kalnauwakul et al. 2007; Leelaporn et al. 2003; Ratchtrachenchai et al. 2004). Most of *E. coli* O157 were incapable of fermenting sorbitol and exhibited colorless colonies on SMAC. Currently, CHROMagar O157 has been developed to differentiate *E. coli* O157 from other *E. coli* strains. *E. coli* O157 appears as mauve colonies on this medium whereas other *E. coli* exhibit blue color colonies (Frampton et al. 1988). Kalnauwakul et al. (2007) also investigated *E. coli* O157 in clinical samples using immunomagnetic separation technique (IMS) followed by cultivation on SMAC and CHROMagar. However, there was no positive result of O157 isolation from this study which may be resulted from the lack of O157 strains in specimens rather than the disadvantage of the technique. Modification of SMAC has been reported by the supplement of cefixime and potassium tellurite into medium (CT-SMAC) to inhibit other bacteria including *E. coli* non-O157 (Zadik et al. 1993). CT-SMAC and CHROMagar in cooperated with enzyme-immunochromatographic assay was applied to isolate *E. coli* O157 in clinical specimens obtained from urban children in Southern Thailand (Voravuthikunchai 2002).

For environmental samples, the use of MacConkey agar followed by DNA hybridization with Shiga-like toxin I (*stx*<sub>1</sub>) and SLT II (*stx*<sub>2</sub>) probes for investigation of

STEC from vegetables, meats, cattle and farm animals in Thailand, were employed. The results showed a range of little to moderate of Shiga-like toxin-producing *E. coli* detection from marketed beef (9%) and at slaughter house (8-28%), particularly, high rate of Shiga-like toxin-producing *E. coli* from cattle feces (11-84%). However, the existence of Shiga-toxin-producing *E. coli* was observed in very low amount from chicken and pork, 1 and 1%, respectively (Suthienkul et al. 1990). Vuddhakul et al. (2000) isolated *E. coli* O157 from bovine feces and beef samples. The samples were enriched in *Escherichia coli* (EC) broth containing novobiocin (20 mg/mL) and were subjected to IMS using O157 antibody-coated magnetic beads followed by the confirmation on SMAC and CHROMagar. It was found that 4 of 95 beef samples (4.2%) and 1 of 55 bovine feces samples (1.8%) were positive. In one study, Voravuthikunchai (2002) employed immunochromatographic assay followed by cultivation on CT-SMAC and CHROMagar to isolate *E. coli* O157 in popular food dishes (under-cooked beef) and found that 6 out of 173 (3.5%) food samples were positive for *E. coli* O157. Two years later, Chomvarin et al. (2005) reported the isolation of *E. coli* O157 from food samples collected in Khon Kaen, North-eastern Thailand. The samples were enriched in EC medium and subsequently cultured on SMAC or subjected to IMS and confirmed on SMAC and CHROMagar. A total of 140 *E. coli* isolates from 186 food samples, were screened for diarrheagenic *E. coli*. The results showed that 10 EHEC (2.7%) were observed. Interestingly, five *E. coli* serotype O157 were found. Recently, Sukhumungoon et al. (2011a) employed different enrichment medium as tryptic soy broth supplemented with novobiocin (20 µg/mL) as an enrichment medium instead of EC broth in the initial process of O157 isolation from Thai beef and beef imported from Malaysia to Southern Thailand. In this study, IMS was performed after enrichment. They were able to isolate 21 *E. coli* O157:H7 from beef. This may suggest that tryptic soy broth incorporated with novobiocin can be used for initial cultivation of *E. coli* O157. In addition, high concentration of Shiga toxin 1-producing *E. coli* was detected in Thai beef by the same method above (Sukhumungoon et al. 2011b). Thus, the initial cultivation in suitable broth medium followed by the application of IMS cultivation, including the differentiation of the colonies on proper medium such as SMAC, CT-SMAC, or CHROMagar O157, indeed enhances the possibility to isolate *E. coli* O157 from food samples.

#### *E. COLI* O157 IN CLINICAL SPECIMENS

It was three decades since the first outbreak of *E. coli* O157 in United States. There were no any outbreaks occurred in Thailand to date. Despite the fact that outbreaks of *E. coli* O157 have not been demonstrated in Thailand, many attempts had been put to investigate this *E. coli* serotype, including other important serotypes from clinical specimens. Seriwatana et al. (1988) employed

colony hybridization using SLT I (*stx*<sub>1</sub>) and SLT II (*stx*<sub>2</sub>) DNA probes to determine enteric bacteria including *Shigella dysenteriae* (30 isolates), enteropathogenic *E. coli* isolated from diarrheal infants (96 isolates), *E. coli* obtained from children less than 5 years old with bloody and non-bloody diarrhea (200 and 140 isolates, respectively), between 1980 and 1987. It showed that all of *E. coli* isolates were negative for *stx* but one isolate of *S. dysenteriae* was positive for *stx*<sub>1</sub>. One year later, Brown et al. (1989) reported the use of *stx*<sub>1</sub> and *stx*<sub>2</sub> specific probes to investigate STEC in children less than 5 years of age with bloody diarrhea and infants (less than 6 months of age) with diarrhea from Children's Hospital and Pramongkutklao Hospital in Bangkok, respectively. Four of 54 children with bloody diarrhea as well as 3 of 50 in control children (without diarrhea) were positive for STEC. One strain recovered from child with bloody diarrhea was positive after hybridization with *stx*<sub>2</sub>, phage 933J and EHEC probes whereas one strain obtained from non-diarrhea control hybridized with *stx*<sub>1</sub> probe only. Both strains were non-O157 serotype and displayed cytotoxicity against HeLa and Vero cells. However, STEC was not detected in 115 infants with diarrhea and 119 controls without diarrhea. Thus, it has been concluded in this section that low numbers of Thai children with and without diarrhea, harbour STEC. In addition, bacteriophage 933J may be associated with the presence of *stx* gene.

During investigation of adults with diarrhea in one hospital in Bangkok using DNA hybridization technique, 7 isolates obtained from two patients were positive for *stx*<sub>1</sub>, *stx*<sub>2</sub>, O157 EHEC fimbriae and produced verotoxin. Two isolates detected in two patients were positive for *stx*<sub>2</sub>, O157 EHEC fimbriae and produced verotoxin. Seven isolates derived from two patients were positive for *stx*<sub>2</sub> and produced verotoxin. In addition, 26 isolates obtained from four patients were positive for O157 EHEC fimbriae only (Bettelheim et al. 1990). None of the isolates possessed O157:H7 serotype. Leelaporn et al. (2003) investigated diarrheagenic *E. coli* from 314 stool samples collected from bloody diarrhea, non-bloody diarrhea and normal subjects using multiplex PCR. Two STEC isolates harbored *stx*<sub>1</sub> were detected in a child with non-bloody diarrhea and possess O26:H<sup>-</sup> and O111:H<sup>-</sup> serotypes. Two isolates possessed *stx*<sub>1</sub> and *stx*<sub>1</sub>-*stx*<sub>2</sub>, were identified from a bloody diarrhea woman and their serotypes were O128:H2 and O125:H21. Interestingly, one isolate (*stx*<sub>1</sub>-*stx*<sub>2</sub>) and serotype O157:H7) was detected in a normal control child. All of the STEC isolates were *eaeA* negative but one isolate (*stx*<sub>1</sub><sup>+</sup>-*stx*<sub>2</sub><sup>+</sup>) harbored *hlyA* and was toxic to Vero cells. This indicates that the infections were due to STEC in Thai population although the most of STEC isolates were low virulent. Based on the evidence, there was an O157:H7 strain in the normal child, it is worth to note that this low virulent organism may raise the immunity to protect the child from the more virulent strains.

Between 1996 and 2000, samples taken from 2100 Thai children less than 12 years of age with acute diarrhea



from 15 hospitals across Thailand were investigated for diarrheagenic *E. coli* using multiplex PCR. It was demonstrated that only one out of 2,629 *E. coli* isolates (0.04%), was STEC and possessed *stx*<sub>1</sub> *stx*<sub>2</sub> and *eaeA* (Ratchrachenchai et al. 2004). Additionally, investigation on 493 diarrheal stools in Hat-yai Hospital, Southern Thailand using culture and IMS techniques found no EHEC isolate (Kalnauwakul et al. 2007).

Thus, it was concluded that the incidence of STEC infections in Thailand, including EHEC are very low and none of them seems to result from EHEC serotype O157 although some of the isolates produced cytotoxin to Vero cells. In addition, detection of O157 fimbriae in some isolates and the presence of STEC O157:H7 in a normal child may induce antibody to O157 in some Thai populations which may lead to the lack of O157 outbreaks in Thailand.

#### *E. COLI* O157:H7 IN FOOD

STEC or EHEC is fecal flora of a wide variety of animals including cattle, sheep, goats, pigs and chickens. Therefore, meat is one source of EHEC O157 contamination. Suthienkul et al. (1990) determined STEC from various sources of meat such as pork, beef and chicken using DNA hybridization technique. Five, two and one percent of beef samples were positive for *stx*<sub>1</sub>, *stx*<sub>2</sub> and both *stx*<sub>1</sub>-*stx*<sub>2</sub>, respectively, whereas only 1% of chicken and pork samples were positive for *stx*<sub>1</sub> and *stx*<sub>1</sub>-*stx*<sub>2</sub>, respectively. Although EHEC plasmid was detected in 5 and 1% of beef and chicken samples, respectively, none of the isolate was identified as EHEC O157. In one study, investigation of foods in Khon Kaen province, North-eastern Thailand, 140 *E. coli* isolates were obtained from 186 food samples (Chomvarin et al. 2005). Using multiplex PCR, three and three isolates were identified as EPEC and ETEC, respectively. Surprisingly, five *E. coli* O157 were detected in several type of foods such as fermented pork sausage (1 isolate), sweet coconut pasties (1 isolate), fermented vegetables (1 isolate), quick-fried basil leaf with chicken (1 isolate) and shrimp chili paste mixed rice (1 isolate). However, all those five isolates were negative for *stx* and *eaeA* genes and did not react with H7 antibody. Therefore, they were not virulent strains.

Vuddhakul et al. (2000) examined 95 beef samples sold in Songkhla province, Southern Thailand, 4 isolates of *E. coli* O157:H7/H<sup>-</sup> were isolated. Three and one isolates were positive for *stx*<sub>2</sub> gene and both *stx*<sub>1</sub> and *stx*<sub>2</sub>, respectively. Furthermore, all these 4 strains contained *eae* gene and 60-MDa plasmid. In one study, Voravuthikunchai et al. (2005) investigated *E. coli* O157:H7 in 173 popular food dishes (under-cooked beef) in the south of Thailand, it was found that only one food sample was positive for verotoxin producing *E. coli* and possessed O157 serotype. Thus, these indicate the incidence of contamination of EHEC O157 in food sold in Thailand.

One obvious characteristic of *E. coli* O157:H7 isolated from Thailand is that they are unable to produce or produce low amount of Stx2. This, in part, resulted from the

existence of *q* gene sequence that is highly homologous to *q* gene of phage 21 (*q*<sub>21</sub>) but not from 933W phage (*q*<sub>933</sub>). Koitabashi et al. (2006) elucidated the inability to produce Stx2 in O157 strains isolated from non-clinical sources of Thailand and Japan. These strains carried *stx*<sub>2</sub> but could not produce Stx2 (Stx2-negative strain). In the study, *E. coli* O157:H7 strain Thai-12 isolated from Thai beef was used as a representative of Stx2-negative strain. By using molecular techniques, *stx*<sub>2</sub> promoter and the *q*-*stx*<sub>2</sub> nucleotide sequences was analyzed. It was found that *stx*<sub>2</sub> promoter of Thai-12 was not functional although *stx*<sub>2</sub> sequence was normal. *q* gene sequence in Thai-12 showed high homology to *q* gene of phage 21, resulting in weak anti-termination activity against a terminator. Therefore, *stx*<sub>2</sub> gene which was located downstream of the terminator could not strongly be transcribed. LeJeune et al. (2004) demonstrated the relationship between types of *q* genes and virulence of *E. coli* O157. Virulent *E. coli* O157 strains isolated from human tend to possess *q*<sub>933</sub> type while the non-virulent strains isolated from bovine tend to possess *q*<sub>21</sub>. Hence, *q*<sub>933</sub> gene was proposed be used as a genetic marker for virulent *E. coli* O157:H7.

#### *E. COLI* O157 IN IMPORTED BEEF

Southern part of Thailand shares border with Malaysia. Population movements, international trades and services have been exchanged across this international border. Food-borne pathogens were reported the second rank in causing disease in this area (Minami et al. 2010). Son et al. (1998) investigated the presence of *E. coli* O157:H7 in retail beef imported from India to Malaysia. It was found that 12 of 63 *E. coli* strains (isolated from 9 of 25 beef samples) were O157:H7. Such strains were EHEC (5 contained *stx*<sub>2</sub>, 7 contained both *stx*<sub>1</sub> and *stx*<sub>2</sub>). Using reverse passive latex agglutination (RPLA) assay, all strains were able to produce Stx. Subsequently, Sahilah et al. (2010) screened the retail beef sold in Serdang, Selangor, Malaysia for *E. coli* O157:H7 carrying *stx* genes. They harvested 20 *E. coli* O157 strains and found that 14 of 20 contained both *stx*<sub>1</sub> and *stx*<sub>2</sub>, while five isolates contained *stx*<sub>1</sub> only and one did not contain *stx* genes. As mentioned before, Malaysia and Thailand shares border, the beef can serve as a vehicle for the transmission of the diseases. Thus, beef imported from Malaysia to Thailand as well as local Thai beef were investigated. Sukhumungoon et al. (2011a) has screened *E. coli* O157:H7 from beef samples during March to September, 2008. Thirty one beef imported from Malaysia and 36 domestic Thai beef samples were collected and examined. Fourteen strains of *E. coli* O157:H7 were isolated from eight Malaysian samples (25.8%) and six strains from four Thai samples (11.1%). Genotype of all strains was *stx*<sub>1</sub><sup>-</sup> *stx*<sub>2</sub><sup>+</sup> *eae*<sup>+</sup> except one Malaysian strain which the genotype was *stx*<sub>1</sub><sup>-</sup> *stx*<sub>2</sub><sup>-</sup> *eae*<sup>+</sup>. All 19 *stx*<sub>2</sub><sup>+</sup> O157:H7 strains produced little or no Stx2. The production of Stx2 from Son et al. (1998) and Sukhumungoon et al. (2011a) was found to be dissimilar. After over ten years of interval, Malaysian *E. coli* O157:H7 demonstrated the incapability of Stx2 production.

TABLE 1. Characteristics of STEC isolated from clinical samples in Thailand during 1989 to 2004

Year	Serotypes	Virulent genes	References
1989	NR <sup>a</sup>	<i>stx</i> <sub>1</sub> or <i>stx</i> <sub>2</sub> alone	Brown et al. 1989
1990	-O2:H1, O110:H19 -O112ab:H21, O113:H21 -O6:H28, O22:H16, O52:H25, O112ab:H21	- <i>stx</i> <sub>1</sub> , <i>stx</i> <sub>2</sub> and O157 fimbriae - <i>stx</i> <sub>2</sub> and O157 fimbriae - <i>stx</i> <sub>2</sub> only	Bettelheim et al. 1990
2003	-O26:H, O111: H, - O128:H2, O125:H21	- <i>stx</i> <sub>1</sub> alone - <i>stx</i> <sub>1</sub> and <i>stx</i> <sub>2</sub>	Leelaporn et al. 2003
2004	-O8	- <i>stx</i> <sub>1</sub> , <i>stx</i> <sub>2</sub> and <i>eaeA</i>	Ratchtrachenchai et al. 2004

<sup>a</sup>NR = No report

For the last decade, *E. coli* O157:H7 Malaysian strains were found to show a high genetic diversity. Sahilah et al. (2010) and Son et al. (1998) who used random amplification of polymorphic DNA (RAPD) to investigate the relationship of the isolates, including the work from Sukhumungoon et al. (2011a) which employed IS-printing and pulsed-field gel electrophoresis as the molecular tools to study genetic relationship among *E. coli* O157:H7 isolates, were consistent. This may suggest that the high rate of genetic change in the Malaysian *E. coli* O157:H7 populations may be resulted from small-size structural polymorphism carried out by insertion sequences (Ooka et al. 2009b).

Even if O157 Malaysian strains were genetically diversified, using pulsed-field gel electrophoresis and O157 IS-printing targeted to IS629, DNA fingerprinting of the O157:H7 strains possessing the *stx*<sub>2</sub> gene demonstrated the close genetic relationship among Malaysian and Thai O157 strains (more than 85%) in one cluster (Sukhumungoon et al. 2011a). Therefore, we discussed for this point that *E. coli* O157:H7 might be transferred from Malaysia to Southern Thailand through beef trade.

#### EHEC BELONGING TO SEROTYPES OTHER THAN O157

Investigation of 10 beef samples in Hat-yai city, Southern Thailand, showed 6 *stx*<sup>+</sup> *E. coli* isolates from four samples (Sukhumungoon et al. 2011b). Four isolates were serotype O157 and possessed the *stx*<sub>2</sub><sup>+</sup> gene whereas the other two strains were non-O157 serotypes possessing *stx*<sub>1</sub><sup>+</sup> and *stx*<sub>2</sub><sup>+</sup>, respectively. Using RPLA assay, the *stx*<sub>1</sub><sup>+</sup> *E. coli* produced high levels of Stx1 (titre = 1: 2,048), but the other five *stx*<sub>2</sub><sup>+</sup> strains were unable to produce Stx2 or produced it in too low amounts to detect (titre < 2). However, serotyping of somatic O antigen and flagella antigen, have not been performed in this strain. Thus, this is the first report of a high concentration of toxin from Stx1-producing non-O157 strain of *E. coli* in Thailand. This strain was able to ferment sorbitol but produced mauve colony on CHROMagar, therefore, it was not different from other EHEC O157 strains and was accidentally picked up. Thus, non-O157 STEC pathogenic strains may be present in the Thai environment but they were overlooked by microbiologists because most laboratories employ immunomagnetic technique using anti-O157 antiserum incorporation of cultivation on SMAC agar to isolate *E. coli* O157:H7.

#### CONCLUSION

In Thailand, incidence of *E. coli* O157 in human samples was very low although the presence of this organism in environmental samples such as cattle, beef and other ready-to-eat foods, has been frequently demonstrated (Chomvarin et al. 2005; Suthienkul et al. 1990; Vuddhakul et al. 2000). These reports pointed to the low or no virulence in Thai O157 strains. Koitabashi et al. (2006) evaluated that some *E. coli* O157:H7 strains obtained in various Asian countries including Thailand possessed a unique *q* gene including surrounding nucleotide sequences which caused them incapable of producing toxin. Therefore, most of *E. coli* O157 obtained in Thailand cannot produce Stx or produce it in a low amount to detect. However, these strains may raise antibody to O157 antigen and protect the hosts to some virulent strains, resulting to non-O157 outbreak reported in Thailand. Nevertheless, STEC other than serotype O157 may play a role in infections on Thai population in an entire time but unfortunately they may be overlooked by researchers because of their characters such as sorbitol fermenter and specific techniques used for O157 detection. Therefore, in Thailand, a search for STEC other than O157 should be undertaken for public health concern.

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